PB87-146163

Evaluation of Benthic Macroinvertebrate Biomass Methodology. Part 1 Laboratory Analytical Methods

(U.S.) Environmental Protection Agency
Washington, DC

1983

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PB87-146163

1. REPORT NO. EPA/600/J-83/356	2.
3. RECIPIENT'S ACCESSION NO.	
4. TITLE AND SUBTITLE: An Evaluation of Benthic Macroinvertebrate Biomass Methodology: Part 1. Laboratory Analytical Methods (Journal Version)	5. REPORT DATE 1983
	6. PERFORMING ORGANIZATION CODE EPA/600/16
7. AUTHOR(S): Mason, William T.; Lewis, Philip A.; and Weber, Cornelius I.	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: U.S. Environmental Protection Agency, Office of Research and Development (RD-682), 401 M Street, S.W, Washington, D.C. 20460	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: U.S. Environmental Protection Agency, Office of Research and Development (RD-682), 401 M Street, S.W., Washington, D.C. 20460	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/16

15. SUPPLEMENTARY NOTES

Environ. Monitoring & Assess., 3:29-44, March 1983

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Analytical Methods Benthic Macroinvertebrate Biomass Assessments Data Index Trophic Pollution Sampling		

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 18
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
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SPRINGFIELD, VA. 22161

EPA/600/J-83/356
JOURNAL ARTICLE

AN EVALUATION OF BENTHIC MACROINVERTEBRATE BIOMASS METHODOLOGY

Part 1. Laboratory Analytical Methods

WILLIAM T. MASON, JR.*, PHILIP A. LEWIS**, and CORNELIUS I. WEBER**

(Received August 1, 1982)

Abstract. Evaluation of analytical methods employed for wet weight (live or preserved samples) of benthic macroinvertebrates reveals that centrifugation at 140 × gravity for one minute yields constant biomass estimates. Less relative centrifugal force increases chance of incomplete removal of body moisture and results in weighing error, while greater force may rupture fragile macroinvertebrates, such as mayflies. Duration of specimen exposure in ethanol, formalin, and formol (formalin -ethanol combinations) causes significant body weight loss with within 48 hr. Formalin and formol cause less body weight loss than ethanol. However, as all preservatives tested cause body weight loss, preservation time of samples collected for comparative purposes should be treated uniformly. Dry weight estimates of macroinvertebrates are not significantly affected by kind of preservative or duration of exposure. Constant dry weights are attained by oven drying at 103 °C at a minimum of four hours or vacuum oven drying (15 inches of mercury pressure) at 103 °C for a minimum of one hour. Although requiring more time in preparation than oven drying and inalterably changing specimen body shape, freeze drying (10 microns pressure, −55 °C, 24 hr) provides constant dry weights and is advantageous for long term sample storage by minimizing curatorial attention. Constant ash-free dry weights of macroinvertebrate samples are attained by igniting samples at 500–550 °C for a minimum of one hour with slow cooling to room temperature in desiccators before weighing.

1. Introduction

Benthic macroinvertebrate communities in unpolluted streams of Temperate North America are often abundant, numbering more than 100 taxa per site. Results of water quality assessments have revealed that streams impacted by organic wastes usually are inhabited by a few tolerant, highly productive taxa, while more diverse, balanced benthic communities inhabit slightly enriched waters. Toxic and acidic substances usually restrict the diversity of communities, and biomass (standing crop in weight per unit area/volume).

Currently, biologists conducting water quality surveys employ the reference station (site) approach, and compare number of individuals per taxon at an unpolluted control site with those inhabiting affected sites. However, rationale for selection of individual counts of animals rather than using weights are not clearly documented. Conventionally, biomass of organisms has been used for studies on the trophic relationships and other expressions of energy flow in aquatic ecosystems.

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Environmental Monitoring and Assessment 3 (1983) 29–44. 0167–6369/83/0031–029$02.40.
1983 *by D. Reidel Publishing Company.*

Analysis of macroinvertebrate biomass potentially represents an inexpensive procedure for water quality assessments, such as analysis of use attainment for waters not expected to meet water quality standards even after implementation of technology based waste load controls. In cases of moderate to extreme organic, toxic, acidic, or solids pollution that involve drastic changes in benthic macroinvertebrate community structure, costly ($300 per sample) counts of individuals and precise identification may be unnecessary. Evaluation of total biomass estimates may yield the same conclusions as individual counts and provide adequate checks for monitoring of the biological health of aquatic ecosystems. Also, weights of benthic taxa may be more intrinsic to characterizations of pollution-caused changes in benthic community structure than are individual counts.

The purpose of this paper is to review past practices for determining macroinvertebrate biomass estimates and to evaluate various alternative methods for use in our study. Data for this paper are part of a comprehensive study on benthic macroinvertebrate methodology including sampling equipment, sample processing, and data interpretive techniques. The study was conducted during 1971 at the East Fork, Little Miami River about 20 miles east of Cincinnati, Ohio, Hamilton, County (see 'Study Site' for details). The time lapse from data collection to reporting does not diminish the data's applicability for today's monitoring programs.

2. Review of Laboratory Analysis Methods

Methods that have been employed to determine weights of invertebrates differ considerably. For live wet weights, Sugden (1967) placed organisms in preweighed, mesh-bottom cans, and drained off excess water for an unspecified period of time. Slack (1967) described a device for measuring small volumetric changes of liquids in a chamber (displacement method) for determining wet weights. Pattee (1968) showed that blot-dried, live, wet weights were less uniform than dry weights, and therefore, he preferred dry weights for biomass estimates. Stanford (1973) removed excess water by centrifugation and reported that the use of different centrifugal forces results in significant differences in the live weights of various macroinvertebrate taxa. He selected a centrifugal force of $52 \times g$ and centrifugation duration of three minutes for sample analysis.

Body length-weight regression equations that take into consideration the size and sex of the animal have been used for obtaining estimates of wet weights (Edmondson, 1971). This technique permits estimates of biomass without destruction of organisms, but unavoidable error may occur in the conversions depending upon differences in morphological characteristics of the species due to geographical variants, instar stage, and environmental conditions. Howmiller (1971) showed that body shrinkage of certain macroinvertebrate taxa increased significantly with duration of exposure in various preservatives. The body shrinkage problem poses potential error in use of length of weight conversions. Also, length-weight measurements require inordinate manpower requirements for routine biomass estimates. Therefore, studies on the variability of biomass estimates based on length-weight equations were not pursued in this study and are not generally recommended for water quality studies.

As with wet weight methods, those for dry weights and ash-free dry weight differ. In an authoritative evaluation of planktonic invertebrate biomass, Lovegrove (1971) showed that drying at 60 °C most closely approximated values obtained by long-term drying in desiccators and suggested that drying at temperatures higher than 80 °C could volatize lipids and remove 'bound' water. He recommended ashing invertebrate tissues at a temperature below 450 °C to avoid loss of bicarbonates.

Powers and Alley (1967) dried organisms at 40–60 °C for 24 hr, and ashed the same at 600 °C for 45 min. Malone and Nelson (1969) dried materials at 90 °C for 24 hr and ashed the samples at 450 °C for 24 hr. Craven and Brown (1969) used oven temperatures of 100 °C and dried to a constant weight (duration not given). Wilhm (1979a) used 103 °C for drying and 500 °C for ashing (no duration given). Crisp (1971) recommended drying temperature at less than 100 °C (no precise temperature given) and ashing tissues at 500–600 °C (4–6 hr) to avoid loss of volatile organic (drying) and to ensure complete destruction of organic matter (ashing). Crisp's recommendations were close to Lovegrove's mentioned above.

Edmondson (1971) in summarizing biomass methodology recounted that 60 °C is the most widely used drying temperature to avoid loss of volatile cellular substances and mentioned the use of freeze drying as a possible alternative method to oven drying.

Paterson (1982) adopted drying bivalve shells and flesh at 60 °C for 48 hr related to studies in a New Brunswick lake. Golightly and Kosinsky (1981) dried bivalve flesh at 105 °C for 72 hr to develop correlations between shell dimensions and weights of tissues. No rationale for selection of these drying temperatures and durations were given.

The conversion of wet weights of macroinvertebrates to drying weight by use of ratios (*w/w*) for isopods was studied in detail by Khmeleva (1971), who reported a conversion factor of 3.33 for *Idotea*. This factor was much smaller than those for planktonic crustaceans that possess higher content of dry residue. He found that wet-dry ratios were representative if all life stages of the isopod were considered collectively, not each life stage independently.

Macroinvertebrates for this study were collected during the summer of 1971 from the East Fork, Little Miami River, located northeast of Cincinnati, Ohio (Figure 1). On August 23, six replicate, 91.4 cm^2 Ekman bottom grab samples were taken from silt-bottom pools at each of four stations. Also, rock-filled basket samples (Mason *et al.*, 1973), exposed for 2, six-week intervals were collected at each station (Figure 2). Because of the rock bottom and swift current at Stations 1, 2, and 4, the basket artificial substrate samples were fastened to threaded steel rods, which were imbedded in 40 × 25 × 8 cm concrete slabs that rested on the streambed. The slabs were then staked to the stream bottom. The baskets were positioned 30 cm below the water's surface (total depth 60 cm at low flow) for the sampling period lasting from July 19 to August 23, and from August 23 to October 12. During the week of August 15–19, drift net samples (one per station) were collected with rectangular nets (net opening at 15 × 30 cm). The mouth of the net was reinforced with canvas and sewn onto a 1/4 inch metal frame. The net was secured vertically (facing upstream 15 cm below the water's surface) by steel rods driven into the substrate. The net was fastened to the rods by cable clamps.

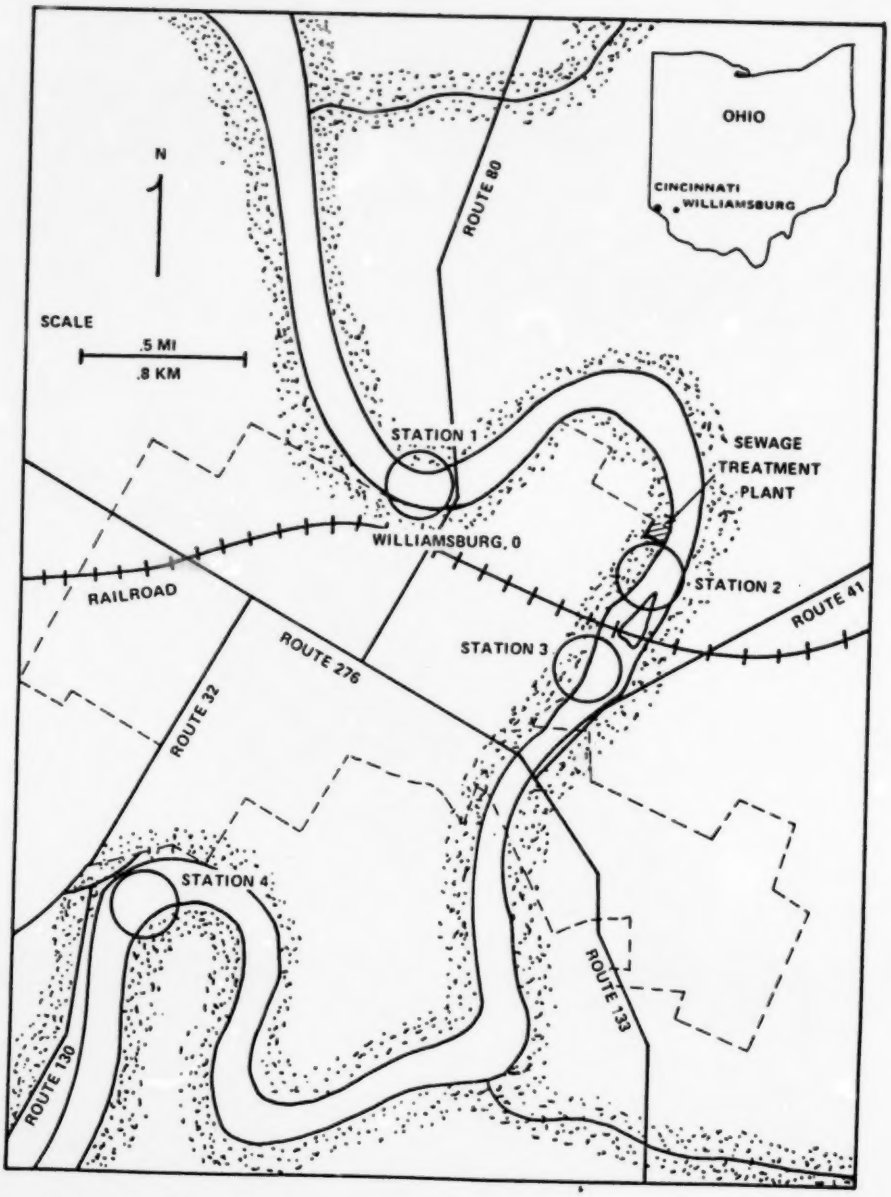

Fig. 1. East Fork of the Little Miami River, Willia.nsburg, Ohio and sampling locations.

Results of benthic sampling and interpretations of various data evaluation techniques will be presented in Part 2.

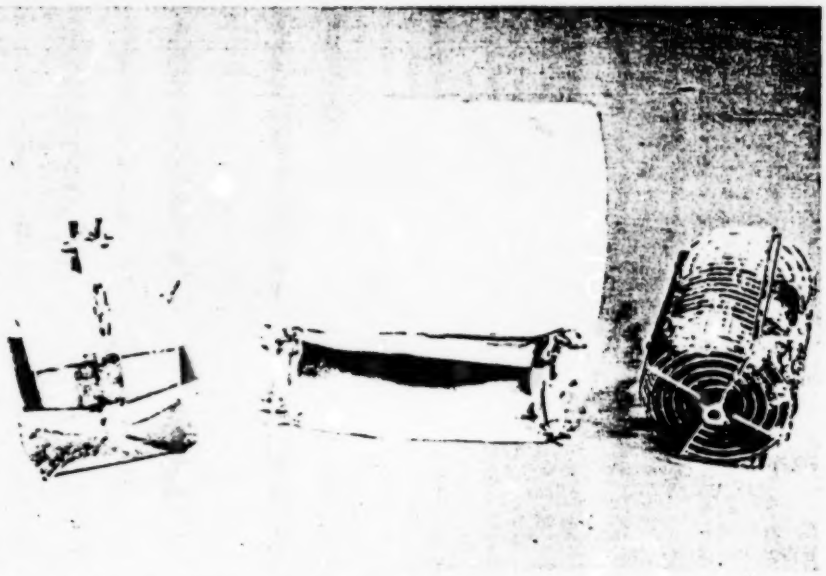

Fig. 2. Sampling gear used for biomass study: (left to right) Ekman Grab, Drift Net, and Rock-filled basket sampler (artificial substrate).

3. Evaluation of Analytical Methods

3.1. Human and instrument error

Each biomass 'analysis' involves a certain degree of human and instrument error in performance of measurements. Before determining biomass estimates for this study, human error and equipment were tested for reproduceability of results (Table I). Human and instrument error (collective) was determined by the technician making eight repetitive weighings (Cahn* Electrobalance) once a day for the same three (22 mm length) freeze-dried chironomid larvae ($< 2\%$ moisture content). After each weighing the specimens were replaced in aluminum foil planchets (Dendy, 1971) and returned to cobalt chloride desiccators. The eight weights ranged from 5.50 to 6.34 mg with mean and standard deviation ($\bar{x} \pm 1$ S.D.) of 6.06 ± 0.25. The Coefficient of Vibration (V = S.D. $\times$ $100/\bar{x}$) for this initial test, 4.12%, was acceptable analytical error, i.e., less than 10%. Other periodic tests for human/mechanical error were performed during the course of the study using different groups of specimens. At no time was human/equipment error greater than 6% of the estimate true sample mean.

* Cahn Instrument Co., Electrobalance Model DTL. Mention of commercial sources does not constitute endorsement by the U.S. Department of the Interior, Fish and Wildlife Service, or U.S. Environmental Protection Agency.

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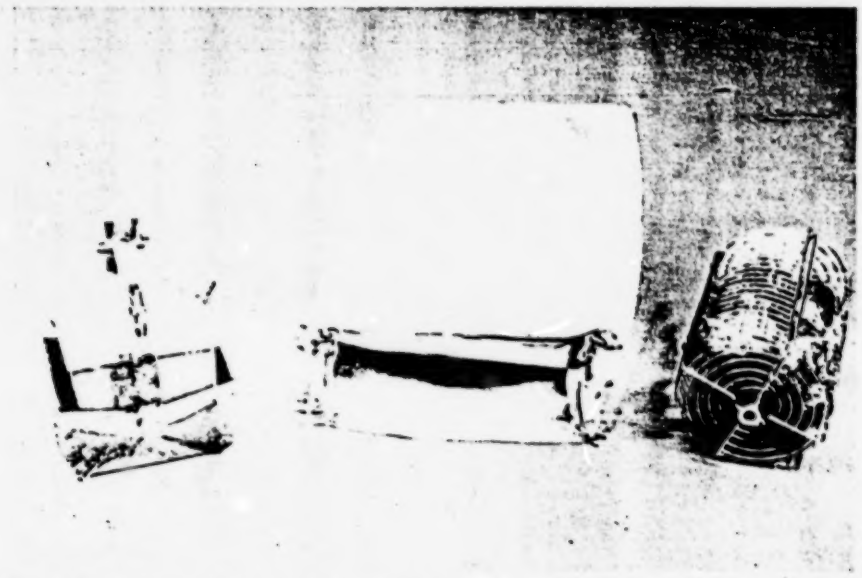

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TABLE I

Design of laboratory analytical methods tests

Treatment	Replicates/set	Taxa/set	Individuals/taxon	Taxon (length mm)
I. Human error effects	1	1	5	*Chironomus* (22)
II. Centrifugation effects	4	4	5	*Hydropsyche* (10)
			5	*Chironomus* (12)
			4	*Stenonema* (7)
			4	*Psephenus* (8)
III. Preservative effects				
A. Wet weights			5	*Cheumatopsyche* (12)
(a) 70% EtOH	0	4	5	*Stenonema* (8)
			3	*Goniobasis* (7)*
(b) 3:1–95% EtOH glacial acetic acid	as in A(a)		3	Elmid Adults (5)
(c) 5% Formalin	as in A(a)			
(d) 1:1–5% Formalin 70% EtOH	as in A(a)			
B. Dry weights				
(a) Control	4	3	5	*Hydropsyche* (9)
			5	*Stenonema* (7)
			5	*Baetis* (4)
(b) 70% EtOH (30 days)	as in B(a)			
(c) 5% Formalin (30 days)	as in B(a)			
IV. Drying temperature and time effects				
A. 40 °C				
(a) 1 hour	4	5	5	*Hydropsyche* (8)
			2	*Stenonema* (9)
			5	*Glyptotendipes* (12)
			3	*Psephenus* (9)
			3	*Goniobasis* (11)
(b) 4 hr	as in A(a)			
(c) 8 hr	as in A(a)			
(d) 24 hr	as in A(a)			
B. 80 °C				
(a) a hour	4	5	5	*Hydropsyche* (8)
			2	*Stenonema* (8)
			5	*Glyptotendipes* (12)
			3	*Psephenus* (8)
			3	*Goniobasis* (11)
(b) 4 hr	as in B(a)			
(c) 8 hr	as in B(a)			
(d) 24 hr	as in B(a)			
C. 103 °C				
(a) 1 hr	4	5	5	*Hydropsyche* (8)
			2	*Stenonema* (8)
			5	*Glyptotendipes* (12)
			3	*Psephenus* (8)
			3	*Goniobasis* (11)

Table I (continued)

Treatment	Replicates/set	Taxa/set	Individuals/taxon	Taxon (length mm)
(b) 4 hr	as in C(a)			
(c) 8 hr	as in C(a)			
(d) 24 hr	as in C(a)			
D. 160 °C				
(a) 1 hr	4	5	5	*Hydropsyche* (8)
			2	*Stenonema* (8)
			5	*Glyptotendipes* (12)
			3	*Psephenus* (8)
			3	*Goniobasis* (11)
(b) 4hr	as in D(a)			
(c) 8 hr	as in D(a)			
(d) 24 hr	as in D(a)			
V. Alternative drying methods				
A. Vacuum oven				
(a) 15 min	4	4	5	*Hydropsyche* (9)
			4	*Stenonema* (7)
			5	*Dicrotendipes* (11)
			4	*Psephenus* (6)
(b) 30 min	as in A(a)			
(c) 60 min	as in A(a)			
(d) 240 min	as in A(a)			
B. Freeze drying				
10μ: −55 °C: 24 hr	4	5	5	*Hydropsyche* (9)
			5	*Stenonema* (7)
			5	*Baetis* (5)
			5	*Glyptotendips* (13)
			2	Elmidae (3)
C. Oven drying 103 °C–4 hr	as in B			
VI. Ash-free dry weights				
A. 250 °C				
(a) 1 hr	4	3	4	*Hydropsyche* (13)
			3	*Stenonema* (9)
			4	*Chironomus* (12)
(b) 4 hr	as in A(a)			
(c) 8 hr	as in A(a)			
B. 350 °C				
(a) 1 hr	4	3	4	*Hydropsyche* (13)
			3	*Stenonema* (9)
			4	*Chironomus* (12)
(b) 4 hr	as in B(a)			
(c) 8 hr	as in B(a)			
C. 450 °C				
(a) 1 hr	4	3	4	*Hydropsyche* (13)
			3	*Stenonema* (9)
			4	*Chironomus* (12)
(b) 4 hr	as in C(a)			
(c) 8 hr	as in C(a)			

Table I (continued)

Treatment	Replicates/set	Taxa/set	Individuals/taxon	Taxon (length mm)
D. 550 °C				
(a) 1 hr	4	3	4	*Hydropsyche* (13)
			3	*Stenonema* (9)
			4	*Chironomus* (12)
(b) 4 hr	as in D(a)			
(c) 8 hr	as in D(a)			

* Measurements for *Stenonema* exclude tail filaments.

3.2. Effects of centrifugation on biomass

The effect of centrifugation on wet weights of macroinvertebrates, i.e., relative centrifugal force (RCF) and duration of RCF was determined for a set of larval macroinvertebrates; *Hydropsyche* (caddisfly), *Chironomus* (midge), *Stenonema* (mayfly), and *Psephenus* (beetle). The centrifugation tubes (2.5 × 15 cm) were fitted with No. 40 mesh wire cones, 2.5 cm deep, to facilitate moisture loss during the centrifugation process. Each replicate within the set (Table I), previously preserved in 70% ethanol for 30 min, were transformed to distilled water for 30 min, blot-dried for 10 sec, weighed (nearest 0.1 mg), and then centrifuged* sequentially at 8.5, 40, 140, and 580 × *g* for one minute. After each weighing the specimens were returned distilled water for 30 min.

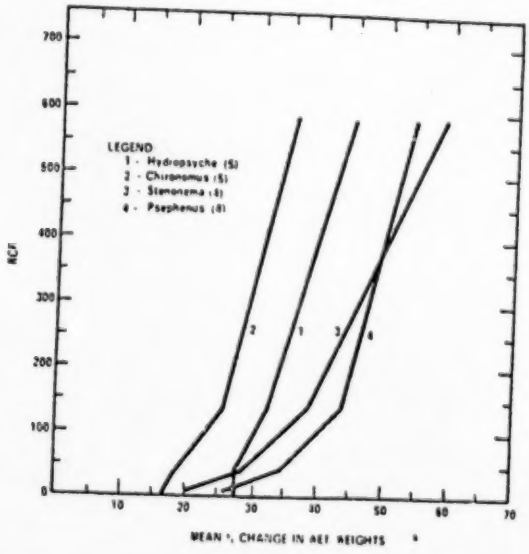

Fig. 3. Mean percent change of weights for larval aquatic insects centrifuged at different relative centrifugal force (RCF).

* International Equipment Co., Model PR-6, Centrifuge 12.4 cm radius.

The mean percent change in wet weight for *Hydropsyche* replicates (Figure 3, curve 1) after blotting and centrifugation at 8.5 × *g* was 27.6 ± 7.0. The difference in the mean percent change on centrifugation at 140 × *g* was not significant $F = 1.37$ ($F.95$: 1,6 d*f* = 5.99). The *F*-value, 20.40 ($F.99$: 1,6 d*f* = 13.7) at 580 × *g* was highly significant (wet weight loss of 44.8%).

The weight loss curve for *Chironomus* sets (Figure 3, curve 2) is similar to that of *Hydropsyche*, and the weight decrease with each successive centrifugation was slight. When centrifuged at forces less than 140 × *g*, *Stenonema* (Figure 3, curve 3) exhibited a similar curve as for *Psephenus* beetle (Figure 3, curve 4). The difference in weight loss between *Stenonema* and *Psephenus* at forces greater than 140 × *g* was caused in part by the loss of *Stenonema's* appendages.

For one minute spin intervals, the wet weight loss by centrifugation increased with increasing centrifugal force. The curves become asymptotic at about 600 × *g*. However, this *g* load ruptures fragile organisms such as mayflies. Thus, for larval aquatic insects, body configuration, sclerotization and overall structural integrity – all greatly influence wet weights obtained by centrifugation methods. For uniform drying and because high *g* loads cause fragmentation, a centrifugal force of about 140 × *g* maintained for one minute was adopted for uniformity in Part 2 methods comparison phase of the study.

3.3. Effects of Preservatives on Biomass

3.3.1. *Wet Weight Test*

Wet weight loss caused by extraction of body substances in various preservatives; 70% ethanol, 3 : 1–95% ethanol and glacial acetic acid, 5% formalin, and 1 : 1–5% formalin and 70% ethanol, was determined for a set of caddisfly *Cheumatopsyche* (caddisfly), *Goniobasis semicarinata* (snail – excluding shell), adult elmid beetles, and *Stenonema* (mayfly). The test was not replicated in four sets as with the above centrifugation experiment. Members of each taxon were weighed live, en masse, centrifuged for one minute at 140 × *g*, placed in the preservatives, and subsequently reweighed after 1, 4, 8, 30, and 55 days following centrifuging at 140 × *g*.

Extrapolation from percent loss of wet weight curves (Figure 4) shows that, except for the scelerotized beetles, the various taxa lost about 30% to 75% of their original live wet weights in ethanol within the first 48 hr after preservation. This corroborates Howmiller's (1971) finding. Most weight stabilized after 96 hr in the preservatives. However, the test for *Stenonema* was suboptimal since the necessary repeated handling of the specimens caused loss of tail filament and legs and caused a disproprortionate wet weight loss compared to the other taxa. Because of body rigidity, the formalin preserved mayflies did not lose as many appendages as the ethanol preserved ones. For *Stenonema* preserved for 96 hr, the wet weight loss was 75% in 70% ethanol; 45% in 5% formalin, 70% in 3 : 1 95% ethanol and glacial acetic acid; and 30% in the formalin-ethanol combination, which produced the least percent wet weight changes in biomass with time.

Soft-bodied *Cheumatopsyche* caddisfly larvae and *Stenonema* mayfly nymphs showed relatively little difference in weight loss using a 1 : 1 mixture of 5% formalin and 70%

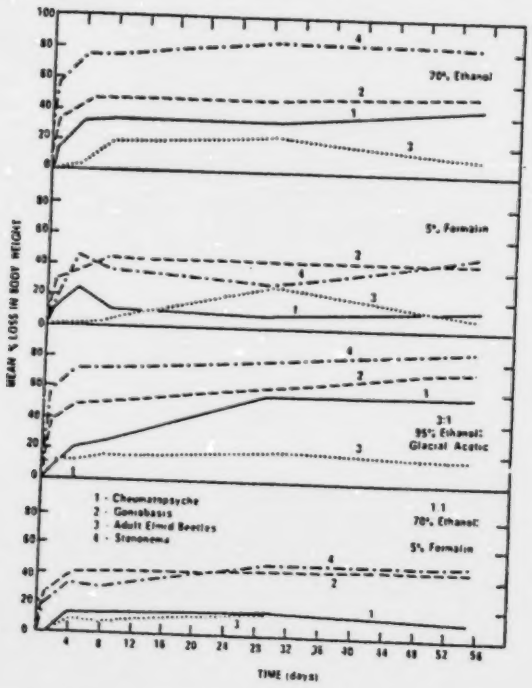

Fig. 4. Percent loss in wet weights of macroinvertebrates in different preservatives.

ethanol and 5% formalin. However, there are major disadvantages to using 5% formalin for routine lab use, including personal sensitivity to the fumes.

As all preservatives tested produced biomass weight loss to varying degrees, any of the preservatives will suffice for station to station sample comparisons.

3.3.2. *Dry Weight Test*

The effects of preservation time on dry weight values were determined for an unpreserved control, 70% ethanol, and 5% (buffered) formalin preserved specimens that were exposed in preservative for 30 days. Five specimens were included in each group (Table I). Genera selected for the test were caddisfly *Hydropsyche*, mayflies *Stenonema* and *Baetis* and consisted of four replicate groups of larvae for each genus.

The specimens, *Hydropsyche* (9 mm length), *Stenonema* (7 mm length), and *Baetis* (4 mm length), were centrifuged live at 140 × *g* for one minute, then weighed and, except for the control, were oven dried in foil planchets at 103 °C for 4 hr. For the 'control' set, live specimens were placed directly into desiccators and cooled to room temperatures before weighing.

For *Hydropsyche* the mean percent change from original wet weight to dry weight for the ethanol preserved specimens was 77.4%, and for the formalin preserved replicates,

TABLE II

Percent change[a] from live wet weights to dry weight for specimens preserved in 70% ethanol and 5% formalin

Taxa	No preservative (control)	Percent change	
		30 days in 70% ethanol	30 days in 5% formalin
Hydropsyche	73.4 ± 1.89	77.4 ± 2.51	78.3 ± 5.43
Stenonema	72.9 ± 1.67	81.2 ± 2.52	88.8 ± 4.43
Baetis	79.5 ± 2.91	91.1 ± 2.12	80.2 ± 4.87

[a] Mean ± 1 S.D. for four replicates, each taxon.

the mean percent change was 78.3% (Table II). Analysis of variance of the control and ethanol mean percent change from preserved wet to dry for biomass of each taxon yielded an F-value of 3.10 ($F.95$: 1,4 df = 7.71) which is not significant. The F-value (3.44) for mean percent change from preserved wet to dry weights for each taxon in the control and formalin sets likewise showed that a preservative time of 30 days did not significantly affect the dry weight estimates.

Wet weight values of preserved organisms are significantly affected by the kind, steeping time, and strength of the preservative, but dry weights are not so affected by preservation factors. Thus, dry weight values offer more consistent, reproduceable results than wet weights for estimating benthic macroinvertebrate biomass.

4. Effects of Drying Temperature and Duration on Biomass

Tests of differences in macroinvertebrate weights due to drying temperatures and duration were conducted using four replicates for each taxon within a set. The taxa included: *Hydropsyche*, *Stenonema*, *Glyptotendipes* (midge), *Psephenus*, and *Goniobasis* (extracted from shells). First, the preserved wet weights (70% ethanol; 48 hr) of the replicates within each set were obtained after centrifugation at 140 × g for one minute. Then each set of specimens was oven dried at 40 °C, 80 °C, 103 °C, and 160 °C for 1, 4, 8, and 24 hr. For example, the 40 °C: 1 hr treatment set consisted of 5 taxa, each replicated 4 times for a total of 20 individual measurements (Table I). As there were 16 possible treatments for the test, the total measurements numbers 320. The sample mean (± 1 S.D.) for each taxon was calculated and used for statistical analysis using a WANG 720 programmable calculator.

Table III shows that the most consistent dry weight values were obtained by drying at 103 °C for at least 4 hr. Two way ANOVA of the mean of means for each treatment shows that drying temperature (40 to 160 °C) is of greater importance (significant at P = 0.01) than time (1–24 hr) in regard to loss of macroinvertebrate body weight; temperature F = 29.4, time F = 2.61 ($F.99$: 3,9 df = 13.3). One-way analysis of variance of the mean percent change detected no significant differences (F = 0.20) between the 40 °C and 80 °C drying temperatures ($F.95$: 1,6 df = 5.99) and 80 °C and 103 °C

TABLE III

Percent change[a] from preserved wet weights to dry weights for five macroinvertebrate taxa at different drying temperatures and times

Drying time (hr)	Mean percent change			
	40 °C	80 °C	103 °C	160 °C
1	69.2 ± 9.76	72.6% ± 7.53	71.9% ± 6.31	77.6% ± 5.37
4	71.7% ± 7.36	69.5% ± 11.59	74.0% ± 6.48	77.9% ± 5.24
8	72.6% ± 7.60	73.3% ± 7.41	74.0% ± 6.61	76.7% ± 6.57
24	72.9% ± 7.46	73.2% ± 7.59	74.1% ± 6.75	78.2% ± 5.12

[a] Mean of means ± 1 S.D.: 4 replicates for each drying temperature and time treatment (see Table I).

temperatures ($F = 1.67$). However, a highly significant F-value of 43.10 ($F.99$: 1,6 $df = 13.7$) occurred between the dry weight values at 103 °C and 160 °C indicating that interstitial water and organic matter not lost at the lower temperature were driven off at some intervening temperature. As constant weights for the groups were achieved after oven drying at 103 °C for four hours, this treatment is preferred for dry weight values when oven drying.

The conversion of macroinvertebrate wet to dry weights has several possible uses including use in water quality indices and production studies. The same macroinvertebrate data used for Table III was used to calculate the wet-dry ratios shown in Table IV.

TABLE IV

Ratio[a] of wet weight to dry weight for macroinvertebrate taxa at different temperatures

Taxon	Oven drying temperature			
	40 °C	80 °C	103 °C	160 °C
Hydropsyche orris	4.50	4.86	4.35	5.42
Stenonema sp.	3.45	3.20	4.34	3.84
Glyptotendipes sp.	6.72	6.68	6.64	7.68
Psephenus sp.	2.68	3.13	2.87	4.26
Goniobasis semicarinata	2.81	3.13	3.10	3.44

[a] Mean of means: 4 replicates for each drying time; 1, 4, 5, and 14 hr (see Table I).

As was demonstrated by two-way ANOVA, drying temperature is the significant factor operating on consistent weight estimates. Therefore, the 1, 4, 8, and 24 hr drying time replicates (4 per taxon for each drying time) were combined to provide the ratios for Table IV. For each taxon, the wet-dry weight ratios were quite consistent, except for the 160 °C temperature. The loss of volatile matter between 103–160 °C affected the ratios (except for *Stenonema*), and therefore, conversion factors for macroinvertebrates are best calculated at less than 103 °C drying temperature.

Two alternate methods for obtaining the dry weight of organisms; vacuum oven and freeze drying, were also examined. Four replicate sets of specimens consisting of groups

of *Hydropsyche* (caddisfly), *Stenonema* (mayfly), *Dicrotendipes* (midge), and *Psephenus* (beetle) were centrifuged live at 140 × *g* for one minute, placed in planchets and weighed. Then, the groups were dried at 15, 30, and 240 min in a vacuum oven* at 103 °C (15 inches of mercury) to determine optimum drying time.

TABLE V

Percent change[a] of macroinvertebrate live wet weights to dry weights for vacuum oven drying (103 °C) at different drying periods

Taxa	Percent change			
	15 min	30 min	60 min	240 min
Hydropsyche	82.6 ± 1.29	83.0 ± 1.26	83.0 ± 1.20	83.4 ± 0.97
Stenonema	80.8 ± 4.14	81.8 ± 3.44	81.7 ± 3.14	81.0 ± 3.30
Dicrotendipes	92.0 ± 1.55	92.2 ± 1.36	92.2 ± 1.38	92.3 ± 1.49
Psephenus	75.3 ± 6.51	75.6 ± 6.46	75.8 ± 6.25	76.8 ± 5.75
$\bar{X}$ % Change	82.7	83.2	83.2	83.4

[a] Mean (± 1 S.D.) for four replicates, each taxon.

The results (Table V) show that for any of the taxa there is little difference in the mean percent changes in weights between 15–240 min. For regular oven drying the percent change from wet to dry weights is 71.9% for the 103 °C–1 hr replicates (Table III) and only the 24 hr–160 °C replicates approximate the 83% wet to dry weight change when using the vacuum oven. It is likely that interstitial water is lost during vacuum oven drying at temperatures higher than 103 °C. This preliminary information suggests that dry weights obtained by regular oven drying are not comparable to weights by vacuum oven drying.

TABLE VI

Percent change[a] of macroinvertebrate live wet weights to freeze and oven dry weights

Taxa	Percent change	
	Freeze-drying	Lven drying
Hydropsyche	77.9 ± 2.03	76.6 ± 1.69
Stenonema	78.8 ± 1.37	79.8 ± 2.33
Baetis	81.0 ± 4.50	74.9 ± 7.59
Elmid Adult	59.1 ± 4.31	61.4 ± 4.43
Glyptotendipes	86.6 ± 0.58	85.6 ± 0.87

[a] Mean (± 1 S.D.) 4 replicates, each taxon (see Table I).

* National Appliance Co., Model 5351 Vacuum Oven.

The mean percent change in weights of freeze-dried* (10 microns pressure, −55 °C, 24 hr) larvae of *Hydropsyche*, *Stenonema*, *Baetis* adult elmid beetle and *Glyptotendipes* did not differ significantly, $F = 0.03$ ($F.95$: 1,8 $df = 5.32$), from weights of individuals oven dried at 103 °C for four hours (Table VI). The larvae were previously preserved in 1 : 1 formol solution, centrifuged dry at 140 × *g* and either oven dried at 103 °C for four hours or freeze-dried (Table I). Each treatment was replicated four times. Freeze drying offers advantages over oven drying because the morphological structures of specimens are not destroyed for later identification purposes, and the samples do not require preservation, once freeze-dried. However, preparation time for this method represents a problem for routine use. In efforts to reduce the drying time, constant weight (2% moisture content) was achieved within four hours by reducing the amount of ice surrounding the specimens to a minimum and increasing the exposed surface area of the ice. Shallow, microbial culture dishes worked best.

4.1. Ash-free dry weights

Optimum temperature and duration for ashing macroinvertebrate samples were determined after first checking the muffle furnace for leaks. Temperatures within the furnace** were found to fluctuate ±25 °C at various locations. Therefore, for uniformity of incinerations, planchets containing the samples were centered on the oven tray. After incineration, the planchets were cooled to room temperature in desiccators before weighing on a Cahn electrobalance. Four sets per treatment (Table I) were prepared consisting of *Hydropsyche*, *Stenonema*, and *Chironomus*, which had been preserved in 70% ethanol for three days, oven-dried at 103 °C for four hours and stored in desiccators. Specimens were weighed in their crucibles and then at 250, 350, and 550 °C for 1, 4, and 8 hr.

Table VII and Figure 5 show that the mean of means percent change from dry weight to ash-free dry weight was less variable at 550 °C regardless of ashing periods. Also Coefficient of Variation (*V*) among replicates was more uniform for the 550 °C treat-

TABLE VII

Percent change* and coefficient of variation (*V*) ash free dry weight for *Hydropsyche*, *Stenonema*, and *Chironomus* at different temperatures and drying periods

Ashing time (hr)	Percent change ashing temperature							
	250 °C	*V* %	350 °C	*V* %	450 °C	*V* %	550 °C	*V* %
1	30.7 ± 8.70	28.23	49.9 ± 2.59	5.19	85.1 ± 8.43	9.90	87.8 ± 4.94	5.63
4	39.5 ± 4.86	12.30	75.7 ± 5.43	7.17	87.2 ± 3.76	4.31	88.3 ± 4.66	5.28
8	45.7 ± 3.25	7.12	80.5 ± 4.08	5.07	89.4 ± 6.09	6.81	88.9 ± 4.06	4.57

* Mean ± 1 S.D.: 4 replicates, for each taxon, temperature, and drying period treatment (see Table I).

* Vertis Co. Freeze Dryer. Mention of commercial source does not constitute endorsement by the U.S. Department of the Interior, Fish and Wildlife Service or U.S. Environmental Protection Agency.
** Lindberg Hevi-Duty Furnace.

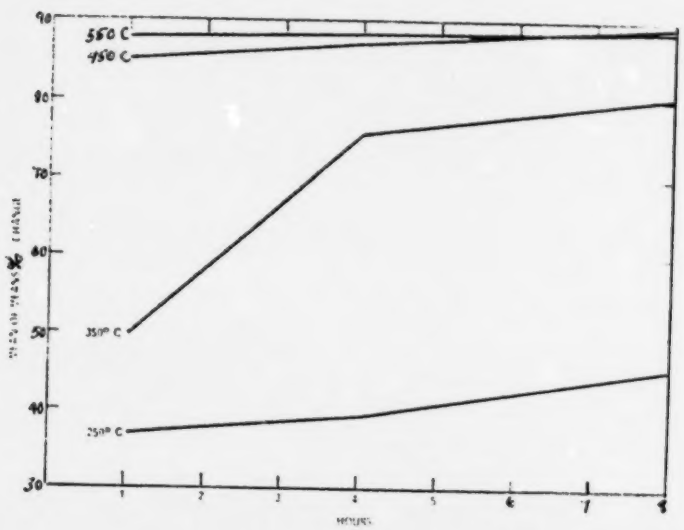

Fig. 5. Percent change of dry weights to ash weights of macroinvertebrates at different temperatures and drying periods.

ment. Two way ANOVA tests for these data provide $F = 30.58$ for 'temperature' (highly significant), and $F = 3.29$ for 'time' indicating that like the drying experiments, temperature is a more important factor than ashing time. Ashing at 550 °C for one hour yields consistent, rapid macroinvertebrate ash-free dry weights.

5. Conclusions

Comparisons of effects of handling on unpreserved and preserved wet weights of various macroinvertebrate taxa reveal that centrifugation at about 140 × gravity provides uniform results without undue fragmentation of specimens, even fragile mayflies. As both ethanol, formalin, and formalin-ethanol preservatives cause significant macroinvertebrate body weight loss within 48 hr, the kind of preservative and time of exposure of specimens in preservatives should be uniform within the same study. Optimum drying to constant weights is obtained by oven at 103 °C for four hours. Freeze-drying offers the advantage of moisture-free drying (2% moisture content) without complete destruction of the specimens, but is more costly and time-consuming than vacuum oven drying. For ash-free dry weights, the optimum ashing temperature and time is 500–550 °C for one hour.

Acknowledgements

The advice of William R. Meehan, U.S. Forest Service, Juneau, Alaska, and Murray G. Johnson, University of Guelph, Ontario, Canada, is gratefully acknowledged. The late Richard P. Howmiller, University of California, Berkeley, provided valuable suggestions concerning the effects of preservatives on weights of macroinvertebrates.

References

Craven, R. E. and Brown, B. E.: 1969, 'Ecology of Hexagenia naiads (Insecta-Ephemeridae) in an Oklahoma Reservoir', *Amer. Midland Natur.* **82**(2), 346–358.

Crisp, D. J.: 1971, 'Energy Flow Measurements', in N. A. Holme and A. D. McIntyre (eds.), *Methods for the Study of Marine Benthos*, International Biological Programme Handbook No. 16. Blackwell Scientific Publications, Oxford, 334 pp.

Dendy, J. S., Pardue, G. B., and Aggus, L. R.: 1971, 'Disposable Planchets for Weighing Macrobenthos', *Prog. Fish Cultl.* **33**(3), 184.

Edmondson, W. T.: 1971, 'Methods for Processing Samples and Developing Data', in W. T. Edmondson and G. G. Winberg (eds.), *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters*, International Biological Programme Handbook No. 17. Blackwell Scientific Publications, Oxford, 358 pp.

Golightly, C. G., Jr., and Kosinski, R. J.: 1981, 'Estimating the Biomass of Freshwater Mussels (Bivalvia:-Unionidae) from Shell Dimensions', *Hydrobiologia* **80**, 263–267.

Howmiller, R. P.: 1971, 'Effects of some Common Preservatives on the Weight of Tubificid Worms and Chironomid Larvae', University of Michigan. Center for Great Lakes Study, Ann Arbor, 5 pp.

Khmeleva, N. N.: 1971, 'Ratio of Wet to Dry Weight in the Isopod Idotea Baltica Basteri from the Black Sea', translated from: *Gibrobiologicheskig Zhurnal* (Hydrobiological Journal) **7**(2), 95–100.

Lovegrove, T.: 1971, 'The Effects of Various Factors on Dry Weight Values', *Rapp. Cons. Explor.* **153**, 86–91.

Malone, C. R. and Nelson, D. J.: 1969, 'Feeding Rates of Freshwater Snails (Goniobasis clavaeformis) Determined with Cobalt', *Ecology* **50**(4), 728–730.

Mason, W. T., Jr., Weber, I., Lewis, and Julian, E. C.: 1973, 'Factors Affecting the Performance of Basket and Multiplate Macroinvertebrate Samplers', *Freshwater Biology* **3**, 409–436.

Paterson, C. G.: 1982, 'Energy Distribution in Biomass Estimates within a Freshwater Bivalve Community', *Can. J. Zool.* **60**, 2753–2756.

Pattee, E.: 1968, 'Relation entre poids frais et poids sec chez quelques invertebres d'eau douce', *Hydrobiologia* **32**(3–4), 417–420.

Powers, C. F. and Alley, W. F.: 1967, 'Some Preliminary Observations on the Depth Distribution of Macrobenthos in Lake Michigan', Special Rept. 30. Great Lakes Res. Div., Inst. Sci. and Tech., Univ. Michigan, Ann Arbor.

Slack, H. D.: 1967, 'A Brief Survey of the Profundal Benthos Fauna of Lakes in Manitoba', *J. Fish. Res. Bd. Can.* **24**(5), 1017–1033.

Stanford, J. A.: 1973, 'A Centrifuge Method for Determining Live Weights of Aquatic Insect Larvae, with a Note on Weight Loss in Preservatives', *Ecology* **54**(2), 449–451.

Sugden, G.: 1967, 'A Technique for Weighing Live Aquatic Invertebrates', *Limnol. and Oceanogr.* **12**(3), 557.

Sugden, G.: 1970, 'Some Aspects of Structure and Function of Benthic Macroinvertebrate Population in a Spring', *Amer. Midland Natur.* **84**(1), 20–35.

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